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A UNIPARENTALLY INHERITED MUTATION AFFECTING PHOTOPHOSPHORYLATION IN CHLAMYDOMONAS REINHARDI

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Summary: A photosynthetically incompetent mutant strain of Chlamydomonas reinhardi shows a uniparental mode of inheritance, a substantial level of photosynthetic electron transport activities, in whole cells and in a cell free system, but a negligible level of photosynthetic phosphorylation activity in a cell free system.

Mutations affecting partial reactions of photosynthesis in the green alga Chlamydomonas reinhardi have been a useful tool in the elucidation of photosynthetic mechanisms in green plants (1,2). To date, there has been only one report of a mutation that affects the photosynthetic phosphorylation system of this organism. The mutant strain F-54 was reported by Sato et al. (3) to lack photophosphorylation capacity and to possess a non-latent Ca^{++} dependent ATPase activity. Bennoun and Chua (4) reported that F-54 lacked three thylakoid membrane polypeptides, 4.1, 4.2, and 8.1, visible normally in SDS gel electrophoresis patterns and exhibited an increase in delayed luminescence yield when compared to wild type.

We describe in this paper a uniparentally inherited mutation of C. reinhardi that impairs photophosphorylation capacity. In this organism, the uniparentally inherited gene has a high probability of being localized in the chloroplast DNA (5,6,7).

Materials and Methods. The wild type strain 137c of C. reinhardi and a mutant strain lip 10-2 were used. Lip 10-2 was obtained from wild type by induction with ultraviolet light followed by arsenate enrichment (8) and rapid screening procedures (9). Both wild type and mutant strains

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Table I

TETRAD ANALYSIS OF *lip 10-2*

CROSS	SEGREGATION PATTERN	NO. OF TETRADS
lip 10-2(+) X *wt*(-)	4:0 (*lip 10-2*:*wt*)	47
	2:2 (*lip 10-2*:*wt*)	0
	0:4 (*lip 10-2*:*wt*)	1
wt(+) X *lip 10-2*(-)	4:0 (*wt*:*lip 10-2*)	57
	2:2 (*wt*:*lip 10-2*)	0
	0:4 (*wt*:*lip 10-2*)	0

Mating and zygote maturation procedures have been described by Ebersold and Levine (13). Following standard procedures, the zygospores (4 or 8) from each germinating zygote were separated and allowed to form individual colonies in light on solid TAP medium. Later, all colonies were replica plated on both tris-minimal and TAP medium. Those isolates that failed to grow autotrophically in tris-minimal medium were scored as mutants while those that grew on tris-minimal medium were scored as wild type.

were grown at 25°C in liquid shake cultures of tris-acetate-phosphate (TAP) medium (10). Photosynthetic O_2 evolution by intact cells, partial reactions of the photosynthetic electron transport system and photophosphorylation in briefly sonicated preparations were assayed as previously described (11,12). Genetic analysis of the mutant strain *lip 10-2* was carried out using the methods described by Ebersold and Levine (13).

RESULTS

Genetic Analysis. Tetrad analysis of a cross between wild type and *lip 10-2* is shown in Table I. A cross of the mutant (mating type +) with wild type (mating type -) gave 47 tetrads with a 4:0 segregation (mutant:wild type), and 1 exceptional tetrad, with 0:4 segregation (all wild type). The exceptional tetrad could be due to the presence of revertant cells in the parental stock. The reciprocal cross between wild type (mating type +) and the mutant (mating type -) gave 57 tetrads in which all colonies were wild type. In both cases, germination exceeded 95%. Thus the incapacity for autotrophic growth in *lip 10-2* showed a typical uniparental inheritance

Table II

PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES IN wild type AND lip 10-2

	REACTIONS	Electron Transport Activities (μmoles O_2 mg chl^{-1} hr^{-1}) Wild-type	lip 10-2
1.	O_2 evolution (whole cell, $H_2O \rightarrow CO_2$)	128	8 (6%)
2.	O_2 evolution (whole cell, $H_2O \rightarrow$PBQ)	135	62 (46%)
3.	O_2 evolution (sonicate, $H_2O \rightarrow$PBQ)	105	36 (34%)
4.	O_2 uptake (sonicate, $H_2O \rightarrow$MV)	98*	41* (42%)
5.	O_2 uptake (sonicate, Asc-DCIP$\rightarrow$MV)	560	520 (93%)

*DCMU sensitive rates.

Cells were suspended in 0.05M tricine-MES, ph 7.7. For briefly sonicated preparations, the cell suspension was sonicated for 10 sec. at 0°C according to the method of Curtis et al (11). The final reaction mixture of 1.3 ml in each assay contained: 1. Intact cells equivalent to 30 µg chl, 50 µmoles Tricine-MES, pH 7.7, and 10 µmoles $NaHCO_3$; 2. Intact cells equivalent to 30 µg chl, 50 µmoles Tricine-MES, pH 7.7, and 0.75 µmole PBQ; 3. Sonicated preparations equivalent to 30 µg chl, 50 µmoles Tricine, MES, pH 8.1, 0.75 µmole PBQ, and 10 µmoles MeAM; 4. Sonicated preparations equivalent to 30 µg chl, 50 µmoles Tricine, MES, pH 8.1, 0.1 µmole MV, 1.0 µmole KCN and 10 µmoles MeAM; 5. Sonicated preparations equivalent to 30 µg chl, 50 µmoles Tricine-MES, pH 7.8, 5 µmoles Asc., 0.05 µmole DCIP, 0.1 µmole MV, 0.01 µmole DCMU, 1.0 µmole KCN, and 10 µmoles MeAM. Other assay conditions were as described in reference (11).

pattern, first discovered by Sager (14). In view of the possible association of uniparental mutations with chloroplast DNA in this organism (5,6,7), it was of interest to ascertain which partial reaction of photosynthesis is affected in lip 10-2.

Electron Transport Analysis. The electron transport activities of wild type and mutant lip 10-2 are compared in Table II. Photosynthetic O_2 evolution by intact cells of lip 10-2 is less than 6% wild type. However, in the presence of PBQ[1], the rate of O_2 evolution by mutant cells is 46% of the wild type, indicating retention of a substantial level of PSII activity in the mutant cells. Using a briefly sonicated preparation, the rates of electron

1. Abbreviations used are: PBQ, p-benzoquinone; MV, methyl viologen; Asc, sodium ascorbate; DCIP 2,6-dichlorophenol-indophenol; PMS, phenazine methosulfate; MeAm, methylamine; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea.

flow from water to PBQ and MV were 34% and 42% of the wild type, respectively. Lastly, PSI mediated electron flow from an Asc/DCIP couple to MV is nearly identical in briefly sonicated preparations of both wild type and lip 10-2. Thus, the near absence of photosynthetic 0_2 evolution in intact cells of lip 10-2 cannot be accounted for solely by impairment of photosynthetic electron transport from water to the reducing side of PSI. (MV is presumed to interact with the endogenous reductant of PSI.)

Phosphorylation Analysis. The photophosphorylation activities of briefly sonicated preparations of wild type and lip 10-2 are shown in Table III. The rate of cyclic photophosphorylation with PMS as a cofactor in the mutant preparation is less than 7% that of the wild type. Noncyclic photophosphorylation activity, using $K_3Fe(CN)_6$, MV, or PBQ as electron acceptors, was not detectable in mutant preparations. The equivalent cell free preparations from wild type showed significant activity. Thus lip 10-2 is capable of substantial photosynthetic electron transport activity but incapable of photophosphorylation. The very low rate of photosynthetic 0_2 evolution in intact cells of lip 10-2 most probably reflects the extremely low level of photophosphorylation of the mutant. In agreement with this conclusion, light dependent incorporation of (^{14}C) acetate by intact cells was observed in the wild type strain but not in lip 10-2 (data not shown); this activity is a measure of in vivo photophosphorylation.

DISCUSSION

Chloroplast DNA has been implicated as the site of uniparental mutations in Chlamydomonas reinhardi (5,6,7). To date, the majority of uniparental mutations reported involve antibiotic resistance or acetate autotrophy (6). Two exceptions include a report by Bennoun et al (15) of a uniparental mutant deficient in a chlorophyll-protein complex CPI and a report by Chua (16) of a uniparental mutant with a variant thylakoid membrane polypeptide. Gelvin and Howell (17) reported localization of the structural gene for the large subunit of ribulose-1,5-bisphosphate carboxylase in the chloroplast DNA of C. reinhardi,

Table III

PHOTOPHOSPHORYLATION IN wild-type AND lip 10-2

Reactions*	Photophosphorylation (μmoles ATP mg chl^{-1} hr^{-1}) Wild type	lip 10-2
1. PMS cyclic	150-240	<10
2. $H_2O \rightarrow K_3Fe(CN)_6$	50-60	∿ 0
3. $H_2O \rightarrow$ MV	40-70	∿ 0
4. $H_2O \rightarrow$ PBQ	25-30	∿ 0

*Measured in a sonicated cell-free suspension

Assay conditions were as described by Brand et al (12). The reaction mixture (1.5 ml) contained briefly sonicated preparations equivalent to 18 μg chl, 50 μmoles Tricine-MES, pH 7.8, 1 μmole ADP, 3.75 μmoles potassium phosphate, pH 7.5 approximately 0.5 μCi ^{32}Pi, and 0.5 μmole $MgCl_2$. In addition, each assay contained; 1. 0.1 μmole phenazine methosulfate (PMS) and 0.01 μmole DCMU; 2. 1.5 μmoles $K_3Fe(CN)_6$; 3. 0.1 μmole methyl viologen (MV); or 4. 0.75 μmole p-benzoquinone (PBQ).

using hybridization of mRNA for the large subunit to chloroplast DNA. Coen et al (18) reported a similar result using Zea mays. While molecular evidence exists for the localization of photosynthetically important gene(s) in chloroplast DNA, genetic evidence relating chloroplast DNA with photosynthesis has been lacking in this organism. We believe that lip 10-2 is the first case of a uniparental mutation affecting a specific partial reaction of photosynthesis, namely photosynthetic phosphorylation.

REFERENCES

1. Bishop, N.I., (1973) Phytophysiology, 8, 65-96.
2. Levine, R.P., (1974) in Algal Physiology and Biochemistry (Stewart, W.D.P., ed.) pp 424-433, Blackwell Science Publ., Oxford.
3. Sato, V.L., Levine, R.P., and Neuman, J., (1971) Biochim. Biophys. Acta. 253, 437-448.
4. Bennoun, P., and Chua, N., (1976) in Genetics and Biogenesis of Chloroplasts and Mitochondria (Bucher, T.H., Neupoert, W., Sebald, W., and Werner, W., ed.) pp 323-329, North-Holland, Amsterdam.
5. Sager, R. (1972) Cytoplasmic Genes and Organelles, Academic Press, New York.
6. Adams, G.M.W., Vanwinkle-Swift, K.P., Gillham, N.W., and Boynton, J.E., (1976) in The Genetics of Algae (Lewin, R.A., ed) pp 69-118, Univ. Calif. Press, Berkeley.
7. Gillham, N.W. (1978) Organelle Heredity, Raven Press, New York.
8. Togasaki, R.K., and Hudock, M.O., In Preparation.

9. Curtis, V.A., Togasaki, R.K., and San Pietro, A., In Preparation.
10. Gorman, D.S., and Levine, R.P. (1965) Proc. Nat. Acad. Sci. U.S.A. 54, 1665-1669.
11. Curtis, V.A., Brand, J.J., and Togasaki, R.K. (1975) Plant Physiol. 55, 183-186.
12. Brand, J.J., Curtis, V.A., Togasaki, R.K., and San Pietro, A. (1975) 55, 187-191.
13. Ebersold, W.T., and Levine, R.P., (1959) Z. Vererb. 90, 74-82.
14. Sager, R. (1954) Proc. Nat. Acad. Sci. U.S.A. 40, 356-363.
15. Bennoun, P., Girard, J., and Chua, N. H., (1977) Molec. Gen. Genet. 153, 343-348.
16. Chua, N., (1976) in Genetics and Biogenesis of Chloroplasts and Mitochondria. (Bücher, T. H., Neupert, W., Sebald, W., and Werner, W., ed.) pp 323-329, North Holland, Amsterdam.
17. Gelvin, S., Heizmann, P., and Howell, S. H. (1977) Proc. Nat. Acad. Sci. U.S.A. 74, 3192-3197.
18. Coen, D. M., Bedbrook, J. R., Bogorad, L., and Rich, A. (1977) Proc. Nat. Acad. Sci. U.S.A. 74, 5487-5491.